BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

Amendments to the Specification:

Please amend page 13, lines 5-31 and page 14, lines 1-12 as follows:

The identity or location of one or more amino acid residues may be changed or modified to include variants such as, for example, deletions containing less than all of the residues specified for the protein, substitutions wherein one or more residues specified are replaced by other residues and additions wherein one or more amino acid residues are added to a terminal or medial portion of the polypeptide (see Figure 2). These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors. Specifically, examples of the amino acid substitutions of serotype 4, included but not limited to, are as follows: E at position 154 is substituted with K; P at position 155 is substituted with L; G at position 156 is substituted with E; E at position 157 is substituted with K; K at position 181 is substituted with E; D at position 182 is substituted with A; R at position 187 is substituted with Y, H, or L; I at position 194 is substituted with N; E at position 200 is substituted with D; E at position 202 is substituted with D; E at position 209 is substituted with K; K at position 212 is substituted with E; V at position 218 is substituted with L; V at position 220 is substituted with K or E; K at position 221 is substituted with E; N at position 223 is substituted with D or K; P at position 225 is substituted with S, T, or R; D at position 227 is substituted with N; E at position 228 is substituted with K; Q at position 229 is substituted with E, G, or D; K at position 230 is substituted with T; K at position 232 is substituted with N; E at position 235 is substituted with K; A at position 236 is substituted with E; E at position 237 is substituted with K; S at position 240 is substituted with N; K at position 241 is substituted with E; Q at position 242 is substituted with K; K at position 249 is substituted with E; K at position 250 is substituted with N; E at position 257 is substituted with Q or K; A at position 263 is substituted with L; K at position 264 is substituted with E; R at position 265 is substituted with N; R at position 266 is substituted with I; A at position 267 is substituted with K or V; D at position 258 is substituted with T; A at

position 269 is substituted with D; A at position 291 is substituted with T, V, P, G, or X; G at position 294 is substituted with G, A, or E; V at position 295 is substituted with D, or A; P at position 295 is substituted with L or F; L at position 2992999 is substituted with P or Q; P at position 328 is substituted with S; E at position 329 is substituted with G; E at position 340 is substituted with A; K at position 343 is substituted with E or D; E at position 347 is substituted with K; D at position 349 is substituted with A; R at position 354 is substituted with H; E at position 366 is substituted with D; E at position 375 is substituted with K; K at position 378 is substituted with E; E at position 390 is substituted with G; P at position 391 is substituted with S; N at position 393 is substituted with D; V at position 397 is substituted with I; and K at position 408 is substituted with Q.

Please amend page 56, lines 31-33 and page 57, lines 1-6 as follows:

A polypeptide comprising a truncated N-terminal fragment of the CbpA (serotype 4) was generated. Full length CbpA was amplified with PCR primers SJ533 and SJ537, the primers were designed based on the derived N-terminal amino acid sequence of the CbpA polypeptide. 5' forward primer SJ533 = 5' GGC GGA TCC ATG GA(A,G) AA(C,T) GA(A,G) GG 3' (SEQ ID NO:41). This degenerate primer designed from the amino acid sequence XENEG, incorporates both BamHI and NcoI restriction sites and an ATG start codon. 3' reverse primer SJ537 = 5' GCC GTC GAC TTA GTT TAC CCA TTC ACC ATT GGC 3' (SEQ ID NO:42). This primer incorporates a SalI restriction site for cloning purposes, and the natural stop codon from CbpA, and is based on both type 4 and R6x sequence.

Please replace page 57, lines 8-13 with the following text:

PCR product was generated from genomic DNA as a template with primers SJ533 and SJ537 amplified 30 cycles with an annealing temperature of 50EC using High Fidelity™ enzyme (Boehringer Mannheim). The resulting PCR products were purified using QIAquick PCR Purification Kit™ (Qiagen, Inc.) then digested with BamHI and SalI restriction enzymes and

cloned into the pQE30 expression vector (Qiagen, Inc.) digested with BamHI, XbaI, and SmaI restriction enzymes.

Please replace page 58, lines 5-8 with the following text:

All polypeptides were expressed and purified with the Qia Expression System™ (Qiagen) using an E. coli the pQE30 vector. The amino terminus of the His tagged proteins are detected by host and Western analysis using both anti-histidine antibodies and protein specific antibodies.

Please replace page 58, lines 21-32 with the following text:

The 1L pellet was re-suspended in 25 ml 50 mM NaH_2PO_4, 10mM Tris, 6M GuC1, 300mM NaC1, pH 8.0 (Buffer A). This mixture was rotated at room temperature for 30 minutes and sonicated on a (VibraCell Sonicator (Sonics and Materials, Inc., Danbury, CT) using the micro tip, two times, for 30 secs, at 50% Cuty Cycle and with the output setting at 7. The mixture was spun 5 min at 10K in a JA20 rotor and the supernatant removed and discarded. The supernatant was loaded onto a 10 ml Talon™ (Clonetech, Palo Alto, CA) resin column attached to a GradiFrac System™ (Pharmacia Biotech, Upsala, Sweden). The column was equilibrated with 100 ml Buffer A and washed with an additional 200 ml of this buffer. A volume based pH gradient using 100% 50 mM NaH_2PO_4, 8M Urea, 20mM MES, pH6.0 (Buffer B) as the final target buffer was run over a total volume of 100 ml. Protein eluted at ~30% Buffer B. Eluted peaks were collected and pooled.

Please replace page 64, lines 6-22 with the following text:

CbpA can be purified over a choline affinity column from its natural host, the pneumococcus~~, as described by Rosenow et al~~. Alternatively, a polyhistidine tag can be engineered onto the end of the gene such that the transcribed protein is extended by several histidine residues. These residues facilitate purification over a nickel affinity matrix Purification of full length

polypeptides as opposed to shorter truncates favors retention of the native tertiary structure. CbpA purified especially from pneumococcus but also from E. coli or other host bacteria by these biochemical means retains its native tertiary structure. Used as an immunogen, natively folded CbpA engenders antibodies that potentially differ from those elicited by immunization with a truncate which may fold differently. Similarly, CbpA used as a therapeutic may have tertiary structure differing from the truncate which would improve its ability to block adherence. Given these considerations, it may be advantageous to produce CbpA as full length protein allowing it to fold to its native tertiary structure and then cleave the C terminal (CBD) away biochemically. For example, treatment with hydroxylamine will cleave CbpA at amino acid position 475 of serotype R6x and of serotype 4 of choline binding protein A, separating the N and C termini. The N terminal fragment is then suitable as a therapeutic or an immunogen.